STUDIES ON THE PHYSIOLOGIC RACES OF *PIRICULARIA ORYZAE* CAV. (II)

by

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The rice blast caused by *Piricularia oryzae* Cav. is one of the most important rice diseases in Taiwan. Hashioka (1950) studying the "mechanism of prevalence" of the disease in the tropics, recognized two major epidemiological types: the temperate-subtemperate and the subtropical-tropical. An epidemic occurrence of the temperate-subtemperate type is determined chiefly by factors related to the growth of the parasite, whereas that of the subtropical-tropical type, chiefly by those related to the resistance of the host. Accordingly, for controlling blast disease in the subtropical and tropical regions emphasis should be laid on the increase of host resistance.

Pathogenic and cultural specialization in rice blast fungus has been studied by several workers. Sasakii (1922-1923) reported three strains of the blast fungus, one being highly virulent and the others less so. Nozu (1928) identified a fourth strain of the fungus. Tochinai and Shimamura (1932) compared 41 cultures of *P. oryzae* and classified them into 9 distinct cultural types. Konishi (1935) studied possible correlation between cultural type and pathogenicity of the fungus, but drew no conclusion. Aoki (1935) described 14 types of *P. oryzae* according to their cultural characteristics. Abe (1936) found marked differences in virulence among strains of the fungus. Inoue (1939) classified isolates into strains on the basis of their ability to decompose cellulose. Comparing the virulence of the fungus by inoculation to leaf and ear of rice, Hemmi (1940) defined 11 strains. Since 1952, Suzuki (1953) has analyzed a large number of single-spore isolates of the blast fungus in an attempt to differentiate them, and established 12 homokaryotic types with respect to ways of spore germination, the ability to form appressoria at different temperatures, etc.. Goto et al. (1960) recently identified races of *P. oryzae* in Japan on the basis of their pathogenicity to selected rice varieties. In America, Latterel et al. (1954) reported the existence of at least two races of *P. oryzae*. In further studies, these authors found fifteen physiologic races characterized by disease reactions in ten rice varieties (Latterell et al., 1960). In 1962, Atkins reported the prevalence and distribution of fifteen pathogenic races of *P. oryzae* in the United States.

Field evidence indicating possible existence of physiologic races in Taiwan

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was first obtained by Li (1957) who observed difference in varietal reaction in blast nurseries established at four locations in 1956. This observation stimulated much research activities on this problem. The study on physiologic races of the blast fungus started in Taiwan Agricultural Research Institute in 1957, and in Academia Sinica, 1960. Hung et al. (1961) employing 16 differential rice varieties recognized five pathologic types among the seven isolates tested. The same group of differential varieties was used in the present studies with 67 isolates collected in 1960, 141 isolates in 1961 and 146 in 1962. Results to be presented here provide a further proof of the existence of physiologic races of *P. oryzae* in this island. The distribution and relative prevalence of the races are also described.

Materials and Methods

Cultures: A total of 354 isolates of *P. oryzae* was obtained from 95 varieties or selections of rice grown in the uniform blast nurseries established in five localities, namely, Ilan, Tungshu, Chiayi, Pingtung and Taitung. The usual tissue plating method was used for obtaining the pure cultures. All cultures were maintained on 2 per cent rice-polish agar slant. Before testing for pathogenicity, the original isolates were subjected to single-spore isolation using the Lambert's cylinder loop needle (Lambert, 1939). A dilute spore suspension was dispersed over the surface of 2 per cent agar plate. After incubating at 28°C for at least 5 hours, the germinating single-spores were cut out and transplanted individually to yeast-starch medium in a vessel (Misato et al., 1961). Spores were usually produced in sufficient numbers in 14 to 18 days for use in plant inoculation.

Differential Varieties: Sixteen varieties of rice used as differentials (Table 1) were selected by the late H. C. Li (1957). These are represented by 11 varieties of *Japonica* type and five varieties of *Indica* type. Of the *Japonica* type, one is from mainland of China, three from Japan and seven locally bred varieties. Of the *Indica* type, two are the upland rice and three the lowland rice.

Table 1. Sixteen rice varieties employed as differentials for identification of physiologic races of *Piricularia oryzae*.

Variety	Type	Origin
Kung-shan-wu-shen-ken	Japonica, lowland	Mainland, China
Taichung 65	Japonica, lowland	Taiwan
Pai-kan-tao	Japonica, lowland	Taiwan
Taichung 171	Japonica, lowland	Taiwan
Chianung 242	Japonica, lowland	Taiwan
Kwang-fu 1	Japonica, lowland	Taiwan
Chianung-yu 280	Japonica, lowland	Taiwan
Taichung line 33	Japonica, lowland	Taiwan

Kanto 51	Japonica, lowland	Japan
Norin 21	Japonica, lowland	Japan
Sensho	Japonica, lowland	Japan
Cutsugulcul	Indica, upland	Taiwan
Natala	Indica, upland	Taiwan
Kao-chio-liu-chou	Indica, lowland	Taiwan
Kaohsiung-ta-li-chen-yu	Indica, lowland	Taiwan
Taichung-ti-chio-wu-chien	Indica, lowland	Taiwan

Growth and inoculation of test plants: Inoculations were made in the green house on 16 differential rice varieties growing separately in an iron flat (30 cm × 30 cm × 7.5 cm). Prior to inoculation, 2% ammonium sulfate solution was top-dressed to the flats twice, at the rate of 25 ml per flat for each application. Two weeks after planting (3–5 leaf stage) the seedlings were inoculated by spraying over leaves with spore suspension which contained 10 to 20 millions of fresh conidia in 100 ml distilled water. Check flats were sprayed with distilled water. Each flat was then kept under plastic sheet on a wooden frame for 20 hours (Anderson 1947). Reading of the disease reactions was made 4 to 9 days after inoculation, depending on the environmental conditions.

Grouping of Infection Types: According to Abumiya (1959), four major infection types of blast disease on rice plants can be recognized on the basis of lesion development and its final appearance.

R type (Resistant): (w)-(p)-b-yb.

M type (Moderately resistant): (w)-(p)-(pg)-bg.

S type (Susceptible): w-p-pg-ybg.

O type: Absence of lesion response.

The symbol ( ) indicates short duration of the described reaction. These systems for grouping infection types were adopted in the present studies except

Table 2. Systems for grouping blast infection types in the present experiment as compared to those adopted elsewhere.

Publisher	Year	Designation							
Hashioka	1950	E	D	C	B		A		
Takahashi	1951	0	1	2	3	5, 6	4		
Abumiya	1953	0	b	yb	bg	ybg	pg	p	w
Abumiya	1959	O	R		M	S			
Goto et al.	1960	HR	R		RS	HS	S		
Latterel et al.	1960	0	1	2	3	4	5		
The present Authors		R			M	S			

that the absence of lesion response was also recorded as R reaction. Somewhat different scales of infection types were proposed by Hashioka (1950), Latterel et al. (1960), Takahashi (1951), and Goto et al. (1960). A comparison of our system with those proposed by others for grouping infection types is given in Table 2.

Experimental Results

Sporulation: Conidia were needed in quantity for use as inocula in pathogenicity tests. It is therefore very important to study the sporulation of the fungus. The sporulability was found to differ with isolates and culture media used. With most isolates, about $60\text{-}80\times10^5$ spores per culture tube were produced on yeast starch agar slant (Yeast extract, 2 g; soluble starch, 10 g; agar, 16 g; water 1,000 ml). Some isolates, however, failed to produce large number of spores on the same medium. Of about 200 isolates tested, 50 yielded more than 5×10^5 spores per tube, 20 formed fewer than 10×10^4, and the rest produced about 9×10^5 spores per tube. Several natural media, e.g., broad-been agar, kindney-bean agar, string-

Table 3. Sporulation of five isolates of *P. oryzae* on different media (pH 5) after two weeks of incubation at 28°C.

Isolate	Medium					
	Yeast-starch agar		Rice-polish agar		Rcie-straw agar	
	Amount*	r**	Amount	r	Amount	r
1Ta-5R	3.00	1.65	2.80	1.63	2.90	1.65
1C-14R	1.40	1.14	0.50	0.70	0.50	0.70
0S-45S	173.40	12.78	28.90	5.33	1.30	3.53
1I-31N	28.60	5.28	2.40	1.47	2.90	1.62
1Ta-5S	134.60	11.19	14.10	3.59	17.30	4.03
L. S. D. 5%		1.701		0.424		0.456
L. S. D. 1%		2.282		0.569		0.612

Isolate	Medium				L. S. D. 5%	L. S. D. 1%
	Rice-seedling agar		String-bean agar			
	Amount	r	Amount	r		
1Ta-5R	1.00	1.00	3.50	1.78	0.233	0.310
1C-14R	0.50	0.70	0.50	0.70	0.339	0.454
0S-45S	6.30	2.42	14.40	3.67	0.745	0.999
1I-31N	16.10	3.97	17.40	4.04	0.570	0.765
1Ta-5S	4.30	1.95	10.60	3.17	1.218	1.634
L. S. D. 5%		0.391		0.582		
L. S. D. 1%		0.525		0.781		

* Average number of spores per 1/30,000 ml.
** Square root of the average number of spores.

bean agar, onion agar, Tochinai-Nakano agar, rice-polish agar, rice-straw agar, etc., also allowed abundant conidial production. With some isolates, these proved to be better media than the yeast starch agar. On corn-meal agar and Czapek's agar very few spores were formed. Table 3 showed the sporulability of several isolates on five different media. Isolates also varied in the length of cultivation that effected spore formation. Most isolates produced considerable amount of spores after one week of incubation at 25-31°C, but a few failed to do so. This is particularly true when yeast starch medium was used. Isolate 0C-5N, for example, produced very few spores after 14 days of cultivation on yeast-starch agar slant at 27-28°C but yielded abundantly after 3 weeks. This phenomenon was not observed with rice-polish agar, however. According to Anderson and Henry (1948), the time required for maximum sporulation may differ with isolates, and with the way in which the cultures are handled. In our results the maximum sporulation was usually obtained after 14 days on yeast-starch agar slant at 27°C by mass mycelial transfer; 4-7 days in vessel containing 40 ml of yeast-starch agar seeded with spore suspension; or 9-12 days in the aerated flask containing autoclaved rice grains. Amount of spores in inoculum was found to be an important factor affecting sporulation, the incubation period required for the maximum production of spores was shortened by using heavier inoculum.

Identification of Races: The 354 isolates tested during 1960-1962 were identified as to belong to 19 physiologic races. Varietal reactions to these races are shown in Table 4. These races can be conveniently distinguished into seven race groups as follows:

Group A: Isolates of this group containing one single race (Race 1) were either avirulent or weakly pathogenic to the 16 differential varieties. No lesions or only resistant lesions were produced under the experimental conditions. Apparently, some isolates of this group might have lost their pathogenicity upon culturing, as evidenced from the fact that they originated from one of the differential varieties and were obtained from susceptible leaf lesions.

Group K: Isolates that constituted this monoracial (Race 2) group caused susceptible leaf lesions only on the variety Kung-shan-wu-shen-ken. No infection or resistant leaf lesions were induced on other varieties. This race group also contained isolates that were obtained from susceptible lesions on differential varieties other than Kung-shan-wu-shen-ken, a fact suggesting the loss of pathogenicity during cultivating.

Group N: This group was represented only by a few isolates of race 3 causing susceptible lesions on the *indica* variety Natala but resistant lesions on other differential varieties. One isolate of this group originally from a susceptible lesion on Taichung 65 failed to induce susceptible reaction on this same variety upon testing.

Table 4. Physiologic races of *P. oryzae* identified in Taiwan during 1960-1962.

Differential variety	Race designation										
	A	K	N	J				I			
	1	2	3	4	5	6	7	8	9	10	11
KSWSK	R	S	R	S	S	S	S	R	R	R	R
TC 65	R	R	R	R	S(m)*	S	R	R	R	R	R
PKT	R	R	R	R	M(r)	S	R	R	R(s)	R	S
TC 171	R	R	R	R	R	R	R	R	R	R	R
CN 242 KF 1	R	R	R	R	R	S	R	S	R	R	R
CNY 280 TCL 33	R	R	R	R	R	R	R	R	R	R	R
KT 51	R	R	R	S	M	S	S	R	R	R	R
NR 21	R	R	R	S	M	S	S	S	R	R	R
SS	R	R	R	S	M	S	S	S	R	S	S
Cuts.	R	R	R	S(m)	S	S	S	R	R	R	S
Nat.	R	R	S	S	S	S	S	S(m)	S	S	R
KSLC	R	R	R	R	S(m)	S	S(m)	S	S(r)	S	S
KH-TLCY	R	R	R	R	M	S	S(m)	S	S	S	S
TC-TCWC	R	R	R	S(m)	R	S(m)	S(m)	S	S	S	S

Differential variety	Race designation							
	P					X		
	12	13	14	15	16	17	18	19
KSWSK	S	S	S	S	S	S	S	S
TC 65	S	S	S	S	S	S	S	S
PKT	S	S	S(m)	S(r)	S	R	S	R
TC 171	S	S	S	S	S	R	R	S
CN 242 KF 1	S	S	S	S	S	R	R	S
CNY 280 TCL 33	S	S	S	S	S	R	R	R
KT 51	S	R	S(r)	R	S	R	R	S
NR 21	S	R	S	R	S	R	R	R
SS	S	R	S(m)	M	S	R	R	R
Cuts.	S(m)	S	S(r)	R	S	R	R	R
Nat.	S(m)	S(m)	S	S	S	R	S(m)	S(m)
KSLC	S	S	S	R	R	R	R	R
KH-TLCY	S	S	S(r)	R	R	R	R	R
TC-TCWC	S	S	M	R	R	R	S	R

* The small letters in parentheses denote the minor infection types observed in the inoculated plants.

Group J: Isolates of four races (4, 5, 6 and 7) that constituted this group produced susceptible leaf lesions on Kung-shan-wu-shen-ken as well as on the two upland *indica* varieties. Lesions of susceptible or intermediate types were induced on the three *japonica* varieties. The four races, however, differed in their reactions on other differential varieties.

Group I: This race group was also composed of four races (8, 9, 10 and 11) which produced susceptible leaf lesions on the three local varieties of lowland *indica* rice, but, resistant reaction on Kung-shan-wu-shen-ken. All or most of the locally developed *japonica* rice varieties responded with resistant reaction.

Group P: Five races (12, 13, 14, 15 and 16) were taken under this group for the susceptible type lesions on Kung-shan-wu-shen-ken and on the seven locally developed *japonica* varieties. The variety Natala from which Taichung line 33 derived its blast resistance also gave susceptible response to these five races. Race 12 of this group was the most virulent one among the 19 races so far recognized. It produced susceptible leaf lesions on all 16 differential varieties.

Group X: This was apparently a highly heterogeneous race group composing of three races (17, 18 and 19) which had few pathogenic properties in common. Susceptible reaction was produced by isolates of the three races on Kung-shan-wu-shen-ken and Taichung 65.

Different races were observed among cultures derived originally from one single-spore isolate. It is not known whether this was due to mutation or intermingling of genetically different nuclei in vegetative hyphae within a heterokaryotic system of the fungus (Suzuki 1962).

Prevalence and Geographical Distribution of Races: The geographical distribution pattern of 19 races of *P. oryzae* identified in Taiwan during 1960-1962 is shown in Table 5.

Races 1, 2, 7, 10, 12, 13, 14, 17 and 18 were obtained from all five areas where blast nurseries were located and collections of the fungus made. Of these races, 13 and 17 seemed to be the most predominant, being isolated over 40 times within the three year period. Races 3, 8, 11 and 19 were found in fewer than four times and confined to two or three localities. Of the 7 race groups, group P could be considered as the most prevalent and widely spread. Races of this group were isolated 121 times and found in all five localities.

The 19 races identfied did not all appear each year during the three year period. The actual number of races detected was 13 in 1960; 12 in 1961; and 17 in 1962. In consideration of locality, 13 races were found in Taitung area; 17 in Pingtung; 15 in Ilan; 14 in Tungshu; 13 in Chiayi. The distribution of the major races in the five areas was as follows: Ilan, races 13 and 17; Tungshu, races 9, 10, 12 and 13; Chiayi, races 12 and 13; Pingtung, races 10, 13, 14, and 17; Taitung, races 12 and 17.

Table 5. Geographical distribution of physiologic races of *P. oryzae* isolated in Taiwan during 1960–1962.

Source	Year	Number of isolates										
		Group A	K	N	J				I			
		Race 1	2	3	4	5	6	7	8	9	10	11
Ilan	1960	1	1									
	1961	2	1		4						1	
	1962	10	2	1			2	2		2		1
Tungshu	1960	3	1		2				2	3	2	
	1961	1	1		2					9	5	
	1962				1		1	1		2	1	
Chiayi	1960										2	
	1961	3	1									
	1962	4	6		2	1		1			1	
Pingtung	1960			1								
	1961	3	1							2	5	
	1962	6	6	1		3	1	2			4	2
Taitung	1960								2			
	1961	1	5							2	2	
	1962	3	2	1	3	2	1	1				
Number of isolates	1960	4	2	1	2	0	0	0	4	3	4	0
	1961	10	9	0	6	0	0	0	0	13	13	0
	1962	23	16	3	6	6	5	7	0	4	6	3
Total		37	27	4	14	6	5	7	4	20	23	3

Source	Year	Number of isolates									Number of races	
		Group P					X			Total		
		Race 12	13	14	15	16	17	18	19			
Ilan	1960	1	1	2			1		1	8	7	
	1961		2	12			1	1		24 } 57	8	15
	1962		1				1	3		25	10	
Tungshu	1960	4	11		1		1			30	10	
	1961	3	6	5			1	7		40 } 80	10	14
	1962	1	1					2		10	8	
Chiayi	1960	2	3	1	1					9	5	
	1961	2	8	3			3	1		21 } 68	7	13
	1962	2	6		1	6	3	5		38	12	
Pingtung	1960	2			3					6	3	
	1961	1	4	1	4		3		2	26 } 71	10	17
	1962		1	2		1	8	2		39	13	
Taitung	1960	2			1		8		1	14	5	
	1961	1	6	2			9	2		30 } 78	9	18
	1962	4		2	4	4	5	2		34	13	
Number of isolates	1960	11	15	3	6	0	10	0	2	67	13	
	1961	7	26	23	4	0	17	11	2	141	12	
	1962	7	9	4	5	11	17	13	0	146	17	
Total		25	50	30	15	11	44	24	4	354	19	

Cultural Characteristics: On yeast-starch medium, six distinct cultural types were discerned among the isolates tested.

Type A: White colony with aerial mycelial growth 1 mm in height, and sparse conidial formation.

Type B: Colony greyish white with aerial mycelial growth 1 mm in height, and sparse conidial formation.

Type C: Colony greyish in color with dark concentric rings, aerial mycelium 2 mm high. Later white tufty oppressed mycelium appearing on the surface. Spores abundant.

Type D: Colony greyish in color with aerial mycelial growth 2 mm in height, and rather profuse sporulation.

Type E: Colony with white coarse aerial mycelial growth 3–4 mm in height and profuse sporulation.

Type F: Colony slightly brownish with mycelial growth 1 mm in height, Sporulation varied from sparse to abundant.

Cultural types of blast fungus were to some extent associated with pathogenicity. However, there was no definite correlation between cultural characters and pathogenic races. Sometimes isolates of different cultural types were pathogenically alike; and sometimes isolates of same cultural type produced different plant reactions in the differentials. Cultures of *Piricularia* sp. isolated from *Panicum repen* (Grass) showed different cultural characteristics from *P. oryzae*. Slower growth was the most conspicious one. Isolates of cultural types C, D and E usually had a strong pathogenicity, while types A and B were weakly pathogenic. Isolates of type F varied from being weak to strong.

Cross-infectivity Tests with P. oryzae and P. sp.: Isolates of *Piricularia oryzae* from rice and *P. sp.* from *Panicum repen* were used to inoculate each other's host. No infection was obtained either on rice by isolates from *P. repen* or on *P. repen* by isolates from rice.

Summary

A total of 354 cultures of *P. oryzae* isolated from 95 varieties or selections of rice grown in five localities of Taiwan were grouped into 6 distinct cultural types according to their cultural characteristics on yeast-starch agar. Using 16 rice varieties as differentials, these isolates were identified as to compose 19 physiologic races which could further be conveniently distinguished into seven race groups on the basis of their ability to attack the differentials. The distribution of the major races in the five areas is as follows: Ilan, Races 13 and 17; Tunshu, Races 9, 10, 12 and 13; Chiayi, races 12 and 13; Pingtung, races 10, 13, 14 and 17; Taitung, races 12 and 17. Of the 7 groups, group P could be considered as the most prevalent and widely spread.

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稻熱病病原菌生理型之研究 (II)

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摘　　要

1. 從1959年起，自全省稻熱病測定試驗病圃（宜蘭、東勢、嘉義、屏東、臺東等地）及臺北、桃園、新竹、臺中、臺南、高雄等地區採集稻熱病病害標本。經人工純系單胞分離後，在酵母培養基上鑒別其培養型，並於溫室內舉行人工接種在十六鑒別稻品種上，以測定各菌株之感病反應。

2. 純系分離之稻熱病病原菌，在酵母培養基上，可分爲6種培養型，以 A, B, C, D, E 及 F 等表之。C, D, E 三型常具强之病原性；A, B 兩型之病原性較弱；F 型之病原性不定。

3. 354 個純系分離之稻熱病病原菌，接種於鑒別稻種上，得到19種不同之生理小種，以 1, 2, 3～19 等表示之。並以品種間感病反應之差異，可組成爲7個小種羣。

4. 19生理小種中，1960年發現13小種；1961年12小種；1962年17小種。宜蘭臺北區共15種生理小種，以13及17最多；臺中東勢區共14種生理小種，以9, 10及13最多；嘉義地區共13種生理小種，以12及13最多；屏東地區共17種生理小種，以10, 13, 14及17最多；臺東地區共18種生理小種，以12及17最多。